Supporting Information

A Fresh Look at Road Salt: Widespread Aquatic Toxicity and Water- Quality Impacts on Local, Regional, and National Scales

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(three total pages, one figure)

Methods

Chloride analyses for Wisconsin samples were done at the using USEPA method 325.2 (1). The method quantification limit was 2.0 mg/L. Average spike recovery during the study period was 100.6% with a standard deviation of 3.3% (n=472). Duplicate analyses resulted in an average relative percent difference of 0.86% with a standard deviation of 1.37% (n = 473).

Toxicity Tests. Pimephales promelas and *Ceriodaphnia. dubia* bioassays were conducted at the Wisconsin State Laboratory of Hygiene in Madison, Wisconsin in accordance with standard U.S. EPA methods (2-4) ((2-4)) and modified U.S. EPA methods ((4)) to determine acute (lethal endpoints) and chronic effects (sublethal endpoints) on the above species. Static renewal acute tests were conducted at 20^{o}C and chronic tests at 25^{o}C. Both were conducted with a 16:8-hour light:dark cycle. Surface-water samples collected during road-salt runoff periods were stored at 4^{o}C upon delivery from the field. Aliquots were removed to prepare test solutions daily. Samples were warmed in a water bath to the appropriate test temperature. Surface-water samples were assayed without dilution.

P. promelas acute tests were initiated with 4-14 day old juveniles. Prior to 2006, each replicate consisted of five fish, which was subsequently increased to ten fish per replicate. The fish were placed in 250 ml plastic cups containing 200 ml of sample. Each treatment consisted of four replicates per sample. Treatment solutions were renewed daily and fish were fed with live brine shrimp two hours prior to the 48-h test renewal. The bioassay was ended at 96-h and survival was recorded as the acute endpoint.

C. dubia acute tests were initiated with young less than 24-h old. Treatments consisted of four replicates per sample containing five *C. dubia* per replicate. Test chambers were 30 ml plastic cups each containing 15 ml sample volume. Test solutions were renewed at 24-h. The *C. dubia* acute test was terminated at 48-h when survival was recorded.

P. promelas chronic growth tests were initiated with <24-hour-old larval fish. Live brine shrimp were fed to the fish three times daily. The tests were terminated on day 7, when the fish were sacrificed, dried, and weighed for determination of growth as the chronic endpoint. In 2000, methods were modified to address mortality due to bacterial pathogens that are commonly found in the study-site streams. Prior to 2000, test treatments consisted of four 250 ml plastic cups, each containing 200 ml of sample and 10 larval fish. Tests were revised after 2000 with 30 ml plastic cups, each containing 25 ml of test solution. Replicates were increased with the method modification from four to ten replicates, with only two fish per test chamber ((4)).

In the *C. dubia* chronic reproduction test, organisms were fed a combination of yeast/cerophyll/trout food and the green algae *Selenastrum capricornutum* with each water renewal. Production of young was recorded daily, and the tests were terminated after 80% of controls released their third brood (6 to 7 days). Test chambers consisted of 30 ml plastic cups, each containing 20 ml of test solution. Each treatment consisted of 10 replicates with one organism per test chamber. The number of young produced was used as the chronic endpoint.

The 25% inhibition concentrations (IC_{25}) were computed using the IC_P method developed by the U.S. Environmental Protection Agency (5).

Fish rearing and toxicity testing were performed following protocols accepted by the Research Animal Resource Center of the University of Wisconsin.

Results

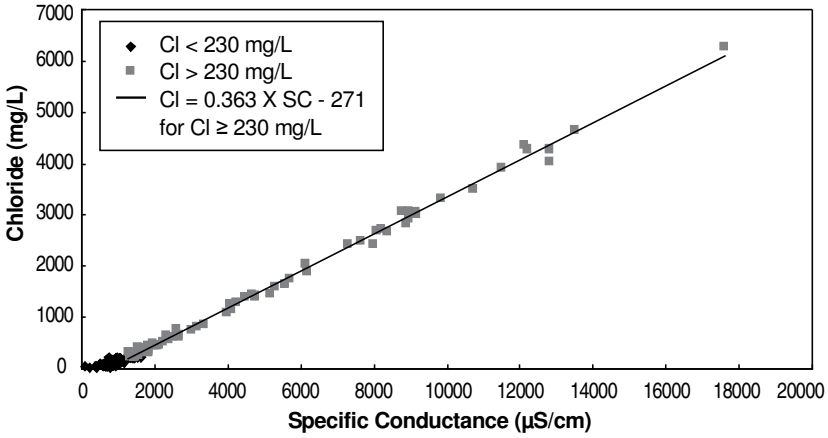

Figure S1. Relation of chloride to specific conductance using data from 17 Wisconsin streams.

References

1. U.S. Environmental Protection Agency *Methods for chemical analysis of water and wastes.* U.S. Environmental Protection Agency: Washington, DC, 1979; Vol. EPA-600/4-79-200.

2. U.S. Environmental Protection Agency *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.* EPA: Washington, DC, 2002;

3. U.S. Environmental Protection Agency *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms.* EPA: Washington, DC, 2002; Vol. EPA-821-R-02-013.

4. Geis, S.W.; Fleming, K.; Mager, A.; Reynolds, L. Modifications to the fathead minnow (Pimephales promelas) chronic test method to remove mortality due to pathogenic organisms. *Environ. Toxicol. Chem.* **2003,** *22,* 2400-2404.

5. U.S. Environmental Protection Agency ICp calculation program, Release 1.0. **1988,**